Anna PARUS[1], Piotr SZULC[2], Zbyszek ZBYTEK[3]
[1] Poznan University of Technology, Institute of Chemical Technology and Engineering,
Berdychowo 4, 60-965 Poznan, Poland
[2] Department of Agronomy, Poznań University of Life Sciences, Dojazd 11, 60-632 Poznań, Poland
[3] Industrial Institute of Agricultural Engineering, Starolecka 31, 60-963 Poznan, Poland
e-mail: anna.parus@put.poznan.pl

EFFECT OF XENOBIOTICS IN THE SOIL ON THE GERMINATION OF MAIZE

Summary

The study focused an assessing the influence of xenobiotics, such as NaCl and diesel oil on germination and early development of maize (Zea mays L.). Through the test the seed germination and growth inhibition of shoot and root of maize were evaluated. The experiments were carried out at different xenobiotics concentration and their mix. It was observed that the presence solely of compounds did not toxic for root growth, but i increase in their concentration caused decreasing of seed germination and root and shoot development. Both sodium chloride and diesel oil were more toxic for shoot and strongly inhibited its growth. The addition of diesel oil to soil contaminated with sodium chloride caused strongest inhibition of seed germination and plant growth.
***Key words**: NaCl, salinity, diesel oil, germination, early seedling growth, maize*

WPŁYW KSENOBIOTYKÓW W PODŁOŻU NA KIEŁKOWANIE KUKURYDZY

Streszczenie

W pracy zostały przedstawione wyniki badań, których celem była ocena wpływu ksenobiotyków, takich jak chlorek sodu i olej napędowy na kiełkowanie i wczesny rozwój kukurydzy (Zea mays L.). Analiza toksyczności związków chemicznych została przeprowadzona na podstawie oceny hamowania kiełkowania nasion oraz wzrostu pędu i korzenia roślin. Doświadczenie przeprowadzono przy różnych stężeniach ksenobiotyków i ich mieszaniny. Stwierdzono, że jedynie obecność małych dawek związków nie jest toksyczna dla kiełkowania i początkowego wzrostu i rozwoju kukurydzy, ale zwiększenie ich stężenia spowodowało obniżenie zdolności kiełkowania nasion oraz rozwoju korzeni i łodyg. Zarówno obecność chlorku sodu, jak i oleju napędowego w glebie były bardziej toksyczne dla łodyg i silnie hamowały ich wzrost. Uprawa kukurydzy w glebie zanieczyszczonej zarówno olejem napędowym jak i chlorkiem sodu wykazywała silniejszą inhibicję kiełkowanie nasion i roślin wzrostu w stosunku do upraw prowadzonych na glebie zawierającej jeden rodzaj zanieczyszczenia.
***Słowa kluczowe**: NaCl, zasolenie, olej napędowy, kiełkowanie, wczesny rozwój roślin, kukurydza*

1. Introduction

The development of civilization, accompanied by industrialization caused a severe contamination of natural environment. The whole biosphere is being degraded due to people destructive activity and live organisms are poisoned by harmful substances from various sources [7, 22, 24]. The advancing industrialization of the word economy has led to a large increasing in the consumption of petrochemical compounds. It also stimulates fuel transport, storage and distribution. As a result, the risk that petroleum substances will permeate into environment has increased [16, 20]. Thus, contamination of natural environment with crude oil and other petroleum substances has become a major issue in ecology. Migration of petroleum substances to water reservoirs, groundwater and soil can have serious consequences due to the highly toxic and cancerogenic nature of such chemicals [15, 18]. Petroleum substances when introduced to environment affect both its abiotic and biotic elements, also microorganism, animals, plants and people.

The seasonal pollutions include deicing products used for winter maintenance treatments. These products have been proven to have adverse effects on groundwater, surface water, soil, vegetation and biodiversity. Large quantities of deicing products are spread every winter in Europe (mainly NaCl, which represents more than 99% of the total consumption) and transferred to roadside environment directly or after passing through various treatment and retention systems (such as constructed wetlands or detention ponds). The salts in water lowers its osmotic potential, resulting in decreased availability of water to the root cell. High salt concentrations could affect various physiological processes in plants [1, 8, 10, 11, 13, 17]. Ratnaker and Rai [12] stated that high salt concentration hampers vital processes such as seed germination, seedling growth and vigour, vegetative growth, flowering as well as fruit set. Thus, high salt concentration ultimately of the produce /???/ [12]. Soil salinity is becoming a serious worldwide problem as more land is irrigated thoroughly and heavily fertilized. Salinity is one of major abiotic stresses in arid and semi-arid regions that substantially reduce the yield of major crops by more than 50%. Salinity affects agricultural production in a large proportion worldwide [3, 14, 23]. Thus, soil contamination with these chemicals is a grave problem as the components of petroleum products and road deicing salts affect all live organisms [2, 10, 11].

In connection with the above, a very interesting study was conducted related to the effect of different NaCl and diesel oil concentrations on germination and seed growth of maize.

2. Materials and methods

Culture was prepared in vertical plastic Phytotoxki™ containers (Phytotoxkit, Tigret company, Belgium). 90 ml of soil were applied to the plates and then a quantity of the analyzed substance (25 ml) was added.The following sets were used in the experiments : (1) control – soil without analyzed compounds, (2) soil contaminated with NaCl (at 0.2, 0.7, 4.0, 8.0, 13.0 $dS \cdot m^{-1}$), (3) soil contaminated with diesel oil (at 0.5, 1.0, 2.5, 5.0, 10.0, 25.0 $ml \cdot kg^{-1}$ dry weight soil (d.w.s.), (4) soil contaminated with diesel oil + NaCl (at 0.5 $ml \cdot kg^{-1}$ d.w.s. diesel oil + 0.2 $dS \cdot m^{-1}$ NaCl; 1.0 $ml \cdot kg^{-1}$ d.w.s. diesel oil + 0.7 $dS \cdot m^{-1}$ NaCl; 2.5 $ml \cdot kg^{-1}$ d.w.s. diesel oil + 4.0 $dS \cdot m^{-1}$ NaCl; 10.0 $ml \cdot kg^{-1}$ d.w.s. diesel oil + 8.0 $ds. \cdot m^{-1}$ NaCl; 25.0 $ml \cdot kg^{-1}$ d.w.s. diesel oil + 8.0 $ds. \cdot m^{-1}$ NaCl; 25.0 $ml \cdot kg^{-1}$ d.w.s. diesel oil + 13.0 $ds. \cdot m^{-1}$ NaCl). Phytotoxkit plastic containers were placed in the dark, constant parameters such as temperature (25 ± 1°C) and humidity (80%) were maintained. On prepared in this way soil, 10 seeds of maize (3 plates for each concentration of the test compounds) were put. At the end of the experiment the number of germinated seeds was counted and the root length and shoot height were measured.

On the basis of obtained results the germination index (GI) for each treatment was calculated using formula:

$$GI = N * L,$$

where: N is the number of germinated seeds and L is the mean root length.

For the experiment, the soil was used having the following elemental composition: 81 mg $P \cdot kg^{-1}$ soil, 88 mg $K \cdot kg^{-1}$ soil, 69 mg $Mg \cdot kg^{-1}$ soil, pH of 5.92 (in KCl), C organic content of 1.01% (10.1 $g \cdot kg^{-1}$ soil), 2.4 mg $N\text{-}NH_4 \cdot kg^{-1}$ d.w.s. and 9.2 mg $N\text{-}NO_3 \cdot kg^{-1}$ d.w.s. Grain size distribution, particle size: 1.0 – 0.1 mm = 95%, 0.1 – 0.02 mm = 5%, < 0.02 mm 0%. Typologically, the field soil was of black earth type, black earth subtype with cambic horizon. The influence of pyridineamidoximes was investigated using the phytotoxicity test based on the ISO-11269-2:2003 International Standard (ISO-11269-2, 2003).

Petroleum diesel oil (EN 590:2004) was purchased from PKN Orlen, Poland. NaCl was obtained from Sigma Aldrich.

In this study seed maize (Zea Mays) purchased from Department of Agronomy in Poznan, Poland was used.

Average values were calculated for each analysed group. All phytotoxicity tests were carried out in three parallels. Average values were evaluated with their standard deviations (SD). Results were plotted with Microsoft Excel software.

3. Results and discussion

The initial germination tests were performed with NaCl and diesel oil and mixture of NaCl and diesel oil (Table 1-3). The control experiments attained 90 to 100% germination on the sixth day after sowing. NaCl did not inhibited germination of maize seed in analysed concentration range. The addition of diesel oil to the soil at concentration of 10 $ml \cdot kg^{-1}$ d.w.s. caused inhibition of seed germination but not more than in 20%. Different situation was observed when soil contained NaCl and diesel oil. Generally, it was observed that germination percentage decreased steadily with increasing NaCl and diesel oil concentration. Already 4 $dS \cdot m^{-1}$ NaCl and 10 $ml \cdot kg^{-1}$ d.w.s. diesel inhibited germination in 30%, and highest concentration 8 $dS \cdot m^{-1}$ NaCl and 25 $ml \cdot kg^{-1}$ d.w.s. diesel and 13 $dS \cdot m^{-1}$ NaCl and 25 $ml \cdot kg^{-1}$ d.w.s. diesel inhibited seed germination in 50%.

Ratnakar and Rai [12], suggested that bigger amount of NaCl might cause disruption of ionic balance in plant cells and also imbalances in plant nutrients which must have affected germination percentages. Mazur [9] also observed negative influences on the germination ability with sodium chloride increasing. This phenomenon is connected with specific ions effect or osmotic stress which later led to reduced water intake into seeds for enhancement of germination. Matrix soil is also a good absorber of xenobiotics and its bioavailability is dependent on solubility and type of soil. The presence of sodium chloride in the medium may affect the availability of xenobiotics to plants.

Table 1. Effect of varying concentrations of NaCl on seed germination of maize
Tab. 1. Wpływ stężenia NaCl na kiełkowanie kukurydzy

	Concentration of NaCl [$dS \cdot m^{-1}$]						
	0	0.1	0.35	2.0	4.0	8.0	13.0
Germination [%]	90.0	100.0	100.0	100.0	90.0	90.0	90.0

Source: own study / *Źródło:* opracowanie własne

Table 2. Effect of varying concentrations of diesel oil on seed germination of maize
Tab. 2. Wpływ stężenia oleju napędowego na kiełkowanie kukurydzy

	Concentration of diesel [$ml \cdot kg^{-1}$ d.w.s.]					
	0	0.5	1.0	2.5	10.0	25.0
Germination [%]	100.0	100.0	100.0	100.0	80.0	80.0

Source: own study / *Źródło:* opracowanie własne

Length measurements of maize root and shoot

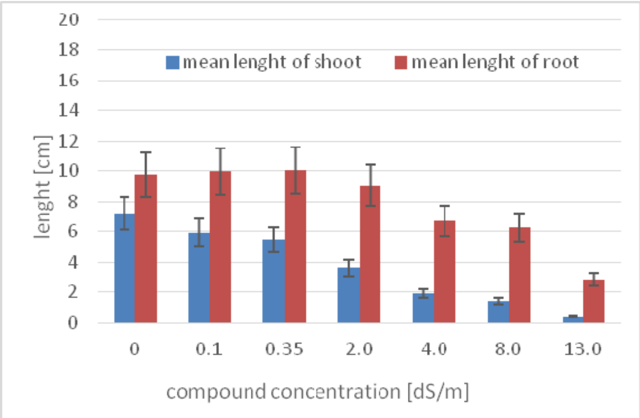
Root and shoot lengths are the most important parameters for studying xenobiotic stress. This is obvious as roots are in direct contact with soil salinity and the effects are then translocated and manifested along the shoot.

The presence of sodium chloride in soil caused that root length in soil with lower NaCl salinity was similar as in control samples, but higher NaCl salinity levels (2.0 $dS \cdot m^{-1}$ and more) affected decrease in root length. Stronger inhibition effect was observed for shoot length. Already NaCl level (0.1 $mS \cdot m^{-1}$) caused corresponding inhibition of shoot length. At the highest NaCl level (13.0 $mS \cdot m^{-1}$) in soil inhibited shoot growth by 80%. Osmotic differences could explain this phenomenon where by lower NaCl concentration positively or not influences solutes to readily cross the cell membranes into the cytoplasm of the cells but in the higher salt concentration, metabolic pumps prevents accumulation of sodium and chloride ions [6].

Table 3. Effect of varying concentrations of mixed NaCl and diesel oil on seed germination of maize
Tab. 3. Wpływ stężenia mieszaniny NaCl i oleju napędowego na kiełkowanie kukurydzy

	Concentration of NaCl + diesel [$dS \cdot m^{-1}$ + $ml \cdot kg^{-1}$ d.w.s.]						
	0	0.1 + 0.5	0.35 + 1.0	2.0 + 2.5	4.0 + 10.0	8.0 + 25.0	13.0 + 25.0
Germination [%]	90.0	100.0	90.0	80.0	70.0	50.0	50.0

Source: own study / *Źródło:* opracowanie własne



Source: own study / *Źródło:* opracowanie własne

Fig. 1. Effect of NaCl concentration on growth parameters of maize

Rys. 1. Wpływ stężenia chlorku sodu na parametry wzrostu kukurydzy

Built up toxic ions emanating from continuous exposure to higher NaCl concentration could lead to decreased availability of some essential nutrients [5]. Another researchers [4] suggested that reduction in plant growth is due to decreasing turgor presser in the soil under saline environment.

Diesel oil was the next analysed xenobiotic. The diesel oil in soil inhibited shoot growth, and the increase in diesel oil concentration decreased shoot length and at highest level (25.0 ml·kg^{-1} soil) shoot length was inhibited in 80%. The root was more resistant on diesel oil. The highest concentration (10.0 ml·kg^{-1} and more) of diesel oil inhibited its length, but not more than in 20%.

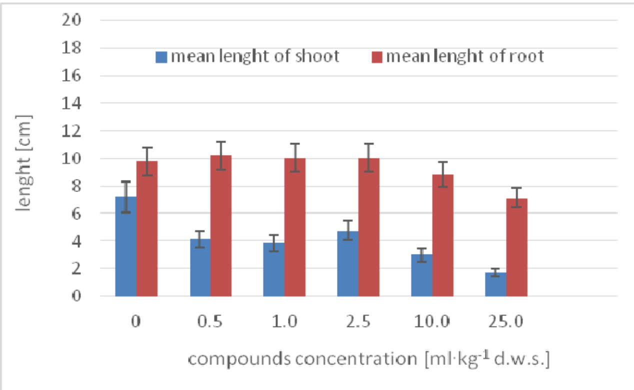


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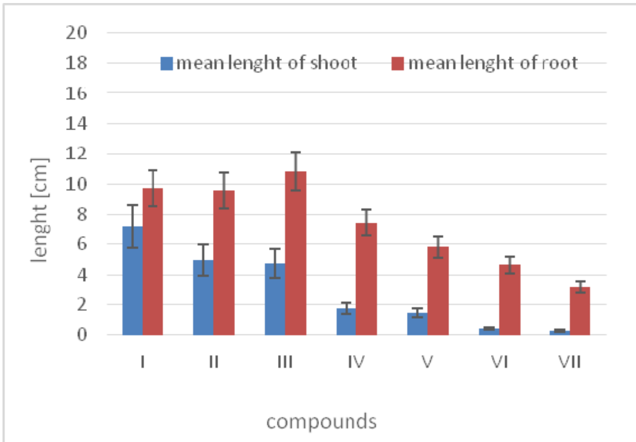
Fig. 2. Effect of diesel concentration on growth parameters of maize

Rys. 2. Wpływ stężenia diesla na parametry wzrostu kukurydzy

Wyszkowski and Ziółkowski [21], who completed experiments with spring barley, spring oilseed rap and yellow lupine as main seed, demonstrated that depressed growth of seeds attributable to soil contamination with petroleum substances retarded the growth of plants proportionately to the contamination. Rates of pollutants even as small as 2.5 cm^3 · kg^{-1} of soil depressed the growth and development of seeds. Regarding spring barley, the highest level of petrol (10.0 ml · kg^{-1} soil) completely stopped plant germination whereas identical rates of diesel oil strongly inhibited the process. Also Wyszkowska and Kucharski [19] confirmed toxic effect of diesel oil on plants, as they demonstrated that it was highly toxic to yellow lupine on light soil when it was introduced to soil in an amount as low as 0.5% of the maximum water capacity. Wyszykowski et al. [20] have also analysed toxicity of petroleum substances towards yellow lupine. They have demonstrated that the extent of the effect produced by soil contamination with diesel oil on the test seed depended on the type of soil, fertilization and plant.

Further experiments were focused on assessing the toxicity of a diesel oil – contaminated soil, which also contained NaCl (Fig. 3). The result of this experiment showed that the presence of these both xenobiotic compounds more inhibited shoot growth than root. The inhibition of shoot length was observed already at lowest concentration (0.5 dS·m^{-1} NaCl and 0.1 ml·kg^{-1} soil diesel oil). The further increasing of compounds concentration in soil caused stronger inhibition of shoot growth, and at concentration 25.0 ml·kg^{-1} soil diesel oil + 8.0 dS·m^{-1} NaCl as well as 25.0 ml·kg^{-1} soil diesel oil + 13.0 dS·m^{-1} NaCl inhibited shoot growth at 94 and 98%, respectively. The presence of NaCl and diesel oil in soil steadily stimulated growth at lower compounds concentration with the best growth stimulation at 0.35 dS·m^{-1} NaCl + 1.0 ml·kg^{-1} soil diesel oil where they achieved highest values respectively. However, roots were adversely affected by higher xenobiotic level (2.0 dS·m^{-1} NaCl + 2.5 ml·kg^{-1} soil diesel oil; 4.0 dS·m^{-1} NaCl + 10.0 ml·kg^{-1} soil diesel oil, 8.0 dS·m^{-1} NaCl + 25.0 ml·kg^{-1} soil diesel oil, 13.0 dS·m^{-1} NaCl + 25.0 ml·kg^{-1} soil diesel oil).

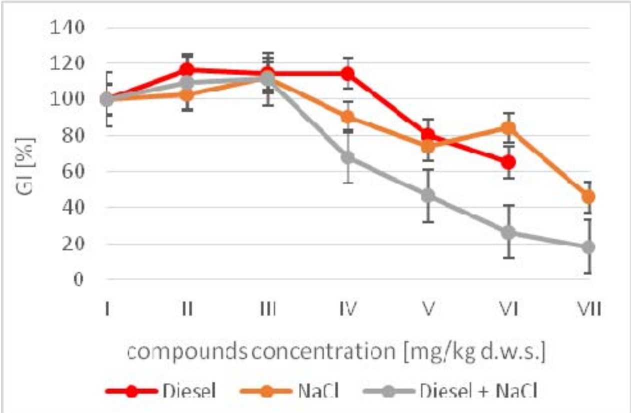


Source: own study / *Źródło:* opracowanie własne

Fig. 3. Effect of mix NaCl with diesel oil concentration on growth parameters of maize (I-0, II-0.5 ml·kg^{-1} d.w.s. diesel oil + 0.2 dS·m^{-1} NaCl; III-1.0 ml·kg^{-1} d.w.s. diesel oil + 0.7 dS·m^{-1} NaCl; IV-2.5 ml·kg^{-1} d.w.s. diesel oil + 4.0 dS·m^{-1} NaCl; V-10.0 ml·kg^{-1} d.w.s. diesel oil + 8.0 dS·m^{-1} NaCl; 25.0 ml·kg^{-1} d.w.s. diesel oil + 13.0 dS·m^{-1} NaCl)

Rys. 3. Wpływ stężenia chlorku sodu i oleju napędowego w mieszaninie na parametry wzrostu kukurydzy(I-0, II-0,5; III-1,0; IV-2,5; V-5,0; VI-10,0; VII-25,0 ml·kg^{-1} d.w.s.; dla gleby zanieczyszczonej dieslem + NaCl: I-0, II-0,5 ml/kg d.w.s. diesel oil+0,2 dS·m^{-1} NaCl; III-1 ml/kg d.w.s. diesel oil + 0,7 dS·m^{-1} NaCl; IV-2,5 ml/kg d.w.s. diesel oil + 4 dS·m^{-1}NaCl; V-10 ml/kg d.w.s. diesel oil + 8 dS·m^{-1} NaCl; 25 ml/kg d.w.s. diesel oil + 13 dS·m^{-1} NaCl)

On the base of the obtained results the germination index (GI) for each treatment was shown in Fig. 4.



Source: own study / *Źródło:* opracowanie własne

Fig. 4. Influence of xenobiotic on the germination index of maize after 6-day germination

Rys. 4. Wpływ ksenobiotyków na indeks kiełkowania kukurydzy po 6 dniach wzrostu

(for soil with NaCl; *dla gleby zawierającej NaCl:* I-0, II-0.2, III-0.7, IV- 4.0, V-8.0, VI-13.0 dS·m^{-1}; for soil contaminated with diesel oil; *dla gleby zanieczyszczonej dieslem*: I-0, II-0.5, III-1.0, IV-2.5, V-5.0, VI-10.0, VII-25.0 ml·kg^{-1} d.w.s.; for soil contaminated with diesel oil + NaCl; *dla gleby zanieczyszczonej dieslem + NaCl*: I-0, II-0.5 ml·kg^{-1} d.w.s. diesel oil + 0.2 dS·m^{-1} NaCl; III-1.0 ml·kg^{-1} d.w.s. diesel oil + 0.7 dS·m^{-1} NaCl; IV-2.5 ml·kg^{-1} d.w.s. diesel oil + 4.0 dS·m^{-1} NaCl; V-10.0 ml·kg^{-1} d.w.s. diesel oil + 8.0 dS·m^{-1} NaCl; 25.0 ml·kg^{-1} d.w.s. diesel oil + 13.0 dS·m^{-1} NaCl ±SD)

The combination of the named xenobiotics contributed to a drop of the GI in the case of maize, which was the most significant at the highest concentration of sodium chloride and diesel oil. The decrease of the GI was most notable for NaCl and mixed xenobiotic (NaCl + diesel oil). Effective concentration, which caused 50% inhibition of root growth was 10.0 ml·kg^{-1} diesel oil + 4.0 dS·m^{-1} NaCl and 13.0 dS·m^{-1} NaCl, respectively mix xenobiotics and sodium chloride.

4. Conclusion

Our studies confirm that the sole presence of xenobiotics or their mix may potentially be toxic to the natural vegetation. The observed toxicity to maize seems to be dependent on type and concentration of xenobiotics. The highest concentration of sodium chloride and also diesel inhibited seed germination and shoot and root development. But higher toxicity was observed for shoot than root. Furthermore, the addition of diesel oil to soil contaminated with sodium chloride caused decreasing of seed germination and plant growth.

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